IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Victor Raso
Prior Application No.: 09/594,366
Prior Application Filing Date: June 15, 2000
Title: IMMUNOLOGICAL CONTROL OF β-AMYLOID LEVELS IN VIVO
Prior Application Art Unit: 1652
Prior Application Examiner: Patterson, C.

EXPRESS MAIL Mailing Label No.: EL848696216US

Date of Deposit with U.S. Postal Service: 11/6/01

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents, Washington, DC 20231.

Tammy L. Moulton
(Typed or printed name of person mailing paper or fee)

Tammy L. Moulton
(Signature of person mailing paper or fee)

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Dear Sir:

Preliminarily, please amend the subject patent application as described below.

<u>In the Claims:</u>

Cancel Claims 1-36 and add new claims 37-82 as shown below.

37. A method for inhibiting the formation of β-amyloid plaques in the brain of a human, the method comprising:
    a) providing a β-amyloid epitope; and
    b) administering the epitope of step a) to the human under conditions appropriate for the stimulation of an immune response directed toward the epitope, the immune response being characterized by the generation of circulating antibodies which bind specifically to the epitope present on endogenous β-amyloid in the human.

38. The method of Claim 37 wherein the epitope of step a) is administered in an adjuvant formulation.

39. The method of Claim 38 wherein the adjuvant formulation comprises an alum adsorption.

40. The method of Claim 38 wherein the adjuvant formulation comprises oil emulsion.

41. The method of Claim 37 wherein the binding of circulating antibodies to endogenous β-amyloid detectably alters the equilibrium distribution of free β-amyloid in circulation versus free β-amyloid in the brain of the human.

42. The method of Claim 37 wherein the epitope of β-amyloid is linked to an immunogenic carrier moiety.

43. The method of Claim 42 wherein the immunogenic carrier moiety is diphtheria toxoid.

44. The method of Claim 42 wherein the immunogenic carrier moiety is hepatitis B core antigen.

45. The method of Claim 37 wherein the epitope is provided as β-amyloid peptide $A\beta_{1-43}$.

46. The method of Claim 37 wherein the epitope is provided as β-amyloid peptide $A\beta_{1-42}$.

47. The method of Claim 37 wherein the epitope is provided as β-amyloid peptide $A\beta_{1-41}$.

48. The method of Claim 37 wherein the epitope is provided as β-amyloid peptide $A\beta_{1-40}$.

49. The method of Claim 37 wherein the epitope is provided as a peptide fragment of β-amyloid, the peptide fragment being derived from the N-terminal region of the β-amyloid peptide $A\beta_{1-43}$.

50. The method of Claim 37 wherein the epitope is provided as a peptide fragment of β-amyloid, the peptide fragment being derived from the central region of the β-amyloid peptide $A\beta_{1-43}$.

51. The method of Claim 37 wherein the epitope is provided as a peptide fragment of β-amyloid, the peptide fragment being derived from the C-terminal region of β-amyloid peptide.

52. The method of Claim 37 which results in the stimulation of an immune response which includes the production of antibodies which bind specifically to immobilized β-amyloid peptides in an *in vitro* binding assay, the immobilized β-

amyloid peptides being selected from the group consisting of: $A\beta_{1-16}$, $A\beta_{14-25}$, $A\beta_{34-43}$, $A\beta_{1-40}$ and $A\beta_{1-43}$.

53. The method of Claim 37 which results in the stimulation of an immune response which includes the production of antibodies which bind specifically to β-amyloid in solution.

54. A method for inhibiting the formation of β-amyloid plaques in the brain of a human, the method comprising:

a) providing a plurality of peptide fragments derived from β-amyloid peptide $A\beta_{1-43}$, each peptide fragment comprising one or more β-amyloid epitopes; and

b) administering the plurality of peptide fragments of step a) to the human under conditions appropriate for the stimulation of an immune response directed toward the β-amyloid epitopes, the immune response being characterized by the generation of circulating antibodies which bind specifically to one or more epitopes present on endogenous β-amyloid in the human.

55. The method of Claim 54 which results in the stimulation of an immune response which includes the production of antibodies which bind specifically to immobilized β-amyloid peptides in an *in vitro* binding assay, the immobilized β-amyloid peptides being selected from the group consisting of: $A\beta_{1-16}$, $A\beta_{14-25}$, $A\beta_{34-43}$, $A\beta_{1-40}$ and $A\beta_{1-43}$.

56. The method of Claim 54 which results in the stimulation of an immune response which includes the production of antibodies which bind specifically to β-amyloid in solution.

57. The method of Claim 54 wherein at least one epitope is provided as a peptide fragment of β-amyloid, the peptide

fragment being derived from the N-terminal region of the β-amyloid peptide $A\beta_{1-43}$.

58. The method of Claim 54 wherein at least one epitope is provided as a peptide fragment of β-amyloid, the peptide fragment being derived from the central region of the β-amyloid peptide $A\beta_{1-43}$.

59. The method of Claim 54 wherein at least one epitope is provided as a peptide fragment of β-amyloid, the peptide fragment being derived from the C-terminal region of β-amyloid peptide.

60. A method for inhibiting the formation of β-amyloid plaques in the brain of a human, the method comprising:
   a) providing an antibody which binds specifically to an epitope of β-amyloid peptide; and
   b) delivering the antibody of step a) into the circulation of the human at concentrations sufficient to detectably alter the equilibrium distribution of free β-amyloid peptide in circulation versus free β-amyloid peptide in the brain of the human.

61. The method of Claim 60 wherein the antibody has the ability to inhibit the formation of β-amyloid plaques.

62. The method Claim 60 wherein the antibody has the ability to disaggregate preformed β-amyloid plaques.

63. The method of Claim 60 wherein the antibody has the ability to hydrolytically cleave β-amyloid.

64. A vaccine composition comprising a β-amyloid epitope in an adjuvant formulation.

65. The vaccine composition of Claim 64 wherein the β-amyloid epitope is linked to an immunogenic carrier moiety.

66. The vaccine composition of Claim 65 wherein the immunogenic carrier moiety is diphtheria toxoid.

67. The vaccine composition of Claim 65 wherein the immunogenic carrier moiety is hepatitis B core antigen.

68. The vaccine composition of Claim 64 wherein the epitope is provided as β-amyloid peptide $A\beta_{1-43}$.

69. The vaccine composition of Claim 64 wherein the epitope is provided as β-amyloid peptide $A\beta_{1-42}$.

70. The vaccine composition of Claim 64 wherein the epitope is provided as β-amyloid peptide $A\beta_{1-41}$.

71. The vaccine composition of Claim 64 wherein the epitope is provided as β-amyloid peptide $A\beta_{1-40}$.

72. The vaccine composition of Claim 64 wherein the epitope is provided as a peptide fragment of β-amyloid, the peptide fragment being derived from the N-terminal region of the β-amyloid peptide $A\beta_{1-43}$.

73. The vaccine composition of Claim 64 wherein the epitope is provided as a peptide fragment of β-amyloid, the peptide fragment being derived from the central region of the β-amyloid peptide $A\beta_{1-43}$.

74. The vaccine composition of Claim 64 wherein the epitope is provided as a peptide fragment of β-amyloid, the peptide

fragment being derived from the C-terminal region of the β-amyloid peptide $A\beta_{1-43}$.

75. The vaccine composition of Claim 64 which results in the stimulation of an immune response which includes the production of antibodies which bind specifically to immobilized β-amyloid peptides in an *in vitro* binding assay, the immobilized β-amyloid peptides being selected from the group consisting of: $A\beta_{1-16}$, $A\beta_{14-25}$, $A\beta_{34-43}$, $A\beta_{1-40}$ and $A\beta_{1-43}$.

76. The vaccine composition of Claim 64 which results in the stimulation of an immune response which includes the production of antibodies which bind specifically to β-amyloid.

77. The vaccine composition of Claim 64 wherein the β-amyloid is free in solution.

78. The vaccine composition of Claim 64 wherein the β-amyloid is aggregated.

79. A method for inhibiting the formation of β-amyloid plaques in the brain of a human, the method comprising:
    a) providing an antibody which binds specifically to an epitope of β-amyloid peptide; and
    b) delivering the antibody of step a) by direct infusion into the brain of the human.

80. The method of Claim 79 wherein the antibody has the ability to inhibit the formation of β-amyloid plaques.

81. The method of Claim 79 wherein the antibody has the ability to disaggregate preformed β-amyloid plaques.

82. The method of Claim 79 wherein the antibody has the ability to hydrolytically cleave β-amyloid.

In the Specification:

Please insert the attached Sequence Listing after page 60 of the specification, and renumber the Claims pages to begin with 64.

Amend the second paragraph of Page 7 to read:

Figure 8 is a structural comparison between the native β-amyloid peptide and the transition state phenylalanine statine β-amyloid peptide analog. β-amyloid peptides shown correspond to amino acids 10-13 of SEQ ID NO: 3.

Amend the third paragraph of Page 7 to read:

Figure 9 is a structural comparison between the native β-amyloid peptide and the reduced peptide bond transition state β-amyloid peptide analog. β-amyloid peptides shown correspond to amino acids 10-13 of SEQ ID NO: 3.

Amend the fourth paragraph of Page 7 to read:

Figure 10 is a formulaic representation of the native C-terminal region of β-amyloid, and the phosphonamidate transition state analog of the C-terminal region of β-amyloid ($A\beta_{35-43}$). β-amyloid peptides shown correspond to amino acids 1-9 of SEQ ID NO: 4.

Amend the fifth paragraph of Page 7 to read:

Figure 11 indicates the putative transition state for peptide hydrolysis by zinc peptidases, compared to the phosphonate and phosphonamidate mimics. The β-amyloid peptide fragments shown for the transition-state and phosphonamidate analog are HCRHNCHR (SEQ ID NO: 6). The peptide fragment shown for the phosphonate analog is HCRCHR (SEQ ID NO: 7).

Amend the sixth paragraph of Page 7 to read:

Figure 12 is a structural comparison of the native β-amyloid peptide and the transition state phosphonamidate β-amyloid peptide which has the peptide link between Gly 38 and Val 39 replaced with a phosphonamidate bond. The β-amyloid peptide shown corresponds to amino acid 4-7 of SEQ ID NO: 4.

Attachment 1

Amended paragraphs with corrections shown

Amend the second paragraph of Page 7 to read:

Figure 8 is a structural comparison between the native β-amyloid peptide and the transition state phenylalanine statine β-amyloid peptide analog. β-amyloid peptides shown correspond to amino acids 10-13 of SEQ ID NO: 3.

Amend the third paragraph of Page 7 to read:

Figure 9 is a structural comparison between the native β-amyloid peptide and the reduced peptide bond transition state β-amyloid peptide analog. β-amyloid peptides shown correspond to amino acids 10-13 of SEQ ID NO: 3.

Amend the fourth paragraph of Page 7 to read:

Figure 10 is a formulaic representation of the native C-terminal region of β-amyloid, and the phosphonamidate transition state analog of the C-terminal region of β-amyloid ($A\beta_{35-43}$). β-amyloid peptides shown correspond to amino acids 1-9 of SEQ ID NO: 4.

Amend the fifth paragraph of Page 7 to read:

Figure 11 indicates the putative transition state for peptide hydrolysis by zinc peptidases, compared to the phosphonate and phosphonamidate mimics. The β-amyloid peptide fragments shown for the transition-state and phosphonamidate analog are HCRHNCHR (SEQ ID NO: 6). The peptide fragment shown for the phosphonate analog is HCRCHR (SEQ ID NO: 7).

Amend the sixth paragraph of Page 7 to read:

Figure 12 is a structural comparison of the native β-amyloid peptide and the transition state phosphonamidate β-amyloid peptide which has the peptide link between Gly 38 and Val 39 replaced with a phosphonamidate bond. The β-amyloid peptide shown corresponds to amino acid 4-7 of SEQ ID NO: 4.

In the Abstract of the Disclosure:

Please cancel the present Abstract of the Disclosure in its entirety, and replace with the following:

> Disclosed are active immunization methods for inhibiting the formation of β-amyloid plaques in the brain of a human. Such methods include the administration of a β-amyloid epitope under conditions appropriate for the stimulation of an immune response directed toward the epitope. The epitope may be present on a length β-amyloid peptide, or a fragment thereof. Disclosed embodiments include the administration of a plurality of such fragments. Also disclosed are methods of passive immunization, as well as vaccine compositions.

REMARKS

Submission of Sequence Listing

The attached paper copy of the Sequence Listing has been prepared in accordance with the provisions of 37 CFR 1.825. Instruction for amendment of the Specification to incorporate the Sequence Listing are provided above.

Also transmitted herewith is a copy of the Sequence Listing in computer readable form. As required by 37 CFR 1.821(f) and (g), Applicants' Attorney hereby states that the content of the Sequence Listing in paper form and on the computer readable form of the Sequence Listing are the same, and the submission includes no new matter.

REMARKS

In light of the above amendments, consideration of the subject patent application is respectfully requested. Please charge any deficiency or overpayment to Deposit Account No. 06-0130.

Respectfully submitted,

Kevin M. Farrell
Attorney for Applicants
Registration No. 35,505
(207) 363-0558

York Harbor, ME 03911
Date: 11\6\07

BBRI\ARC\2005.PA